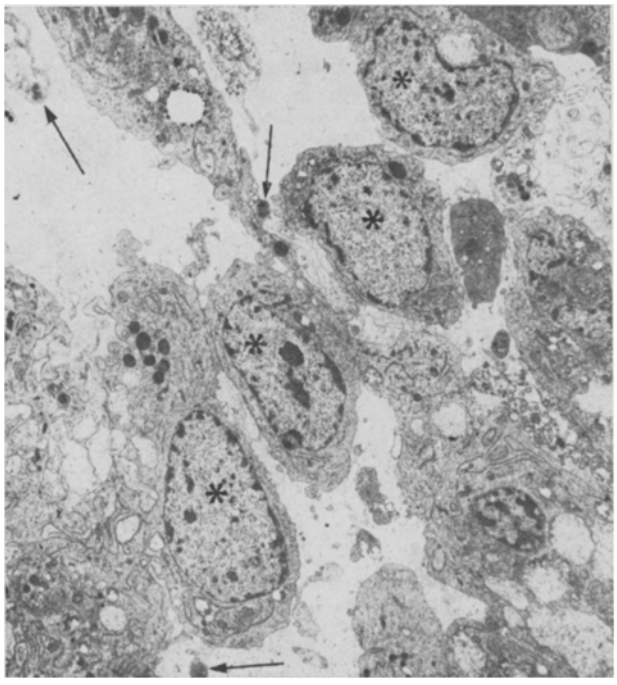

Fig. 1. Stade précoce de la différenciation d'hémocytes granuleux (*) à l'intérieur du tissu hématopoïétique de *Melolontha melolontha*. En bas à gauche et à droite, cellules réticulaires; en haut à gauche, stade avancé de différenciation. Remarquer la présence de fibres caractéristiques (flèches). Fixation au glutaraldéhyde-acide osmique. Coupes contrastées à l'acétate d'uranyle-citrate de plomb. ×2800.

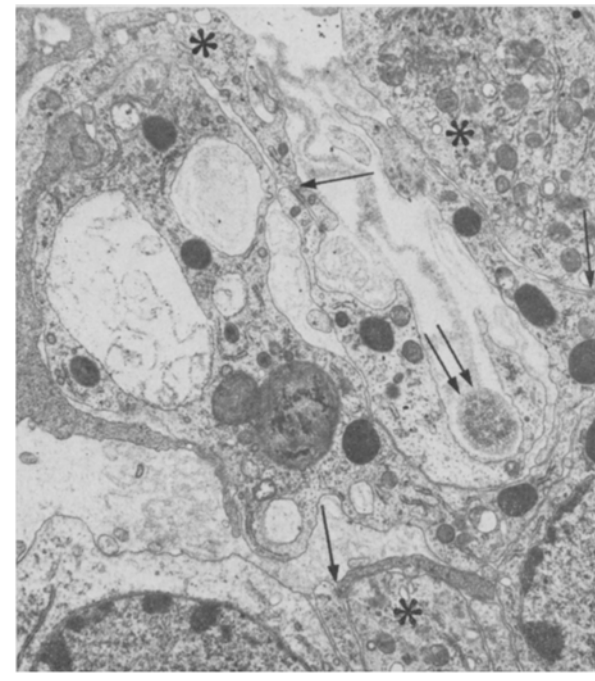

Fig. 2. Organisation fine du tissu hématopoïétique de *Melolontha melolontha*. On observe deux granulocytes en voie de différenciation et des cellules réticulaires (*). Noter l'organisation réticulée des éléments cellulaires et des fibres de matériel basal (avec densification centrale; double flèche). Les cellules présentent entre elles des desmosomes (flèches). Fixation au glutaraldéhyde-acide osmique; coupes contrastées à l'acétate d'uranyle-citrate de plomb. ×8400.

raissent, dans certaines de ces cellules, les inclusions caractéristiques des divers types hémocytaires: granules, sphérules ou corps structurés. Les mitoses sont nombreuses dans ces hémocytes en voie de différenciation, dont la maturation se fait ainsi par îlots d'hémocytes de même type au même stade de différenciation.

L'organisation réticulaire des cordons hémocytaires présents à l'intérieur du sinus péricardiaque de *Melolontha melolontha* permet de faire un rapprochement avec les tissus hématopoïétiques d'autres groupes zoologiques. Les potentialités hématopoïétiques des cellules réticulaires sont démontrées par la différenciation des divers types d'hémocytes dans ce système de cordons. L'activité macrophagique des cellules réticulaires assurant la destruction d'hémocytes agés, renforce l'analogie de ce tissu avec les organes hématopoïétiques des vertébrés.

Il convient en outre de souligner l'analogie entre la structure de l'organe hématopoïétique et le mécanisme de l'hématopoïèse chez *Melolontha melolontha* d'une part et chez *Locusta migratoria*, *Gryllus bimaculatus*, *Gryllotalpa gryllotalpa*[2] et *Calliphora erythrocephala*[3] d'autre part. Il nous semble vraisemblable que ce phénomène présente une grande généralité pour l'ensemble des ordres d'insectes.

Summary. The hemocytopoietic tissue of larvae of *Melolontha melolontha* consists of irregular cell accumulations situated between the large pericardial cells in the dorsal part of the abdomen. The morphological and functional characteristics of this tissue are very close to those described for the hemocytopoietic tissue of other insects (*Locusta migratoria*, *Gryllus bimaculatus* and *Calliphora erythrocephala*) and the differentiation of several blood cell types could also be followed in this tissue.

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Dimethyl Sulphoxide Effect on Division Cells

Dimethyl sulphoxide (DMSO), a lignin-derived chemical, has recently been under study from very different points of view on account of its diversity of properties: as increasing the penetration of substances into animal and plant tissues[1], for its cryoprotective properties[2,3], as a solvent of phytopesticides[4,5] and of hydrophobic organic compounds in general[6,7]. In this paper, we report the effect of DMSO, at different concentrations, on plant cell populations, showing how it affects their kinetics and the general picture of morphological changes to which it gives rise at the cell level.

The material consisted of meristems from the bulb-roots of *Allium cepa* L. growing in tap-water at 25 °C with constant aeration, and subjected to 20, 10, 6 and 1% aqueous concentrations of DMSO. The observations were carried out under the light microscope after fixation of

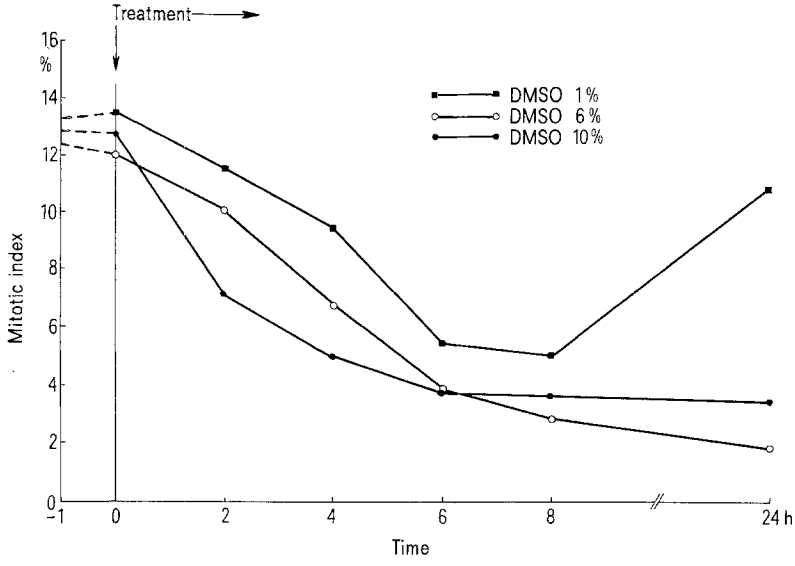

Fig. 1. Mitotic indices before and during the DMSO treatment at different concentrations.

the roots in 3:1 acetic ethanol and staining with acetic-hydrochloric orcein according to the technique of TJIO and LEVAN[8].

The mitotic index[9], expressed as a percentage of the total number of meristematic cells, was determined by examining 500 to 1000 cells form each preparation, with readings from different roots at 2-h intervals in the course of the treatment. The percentage of cells in the different stages of mitosis was calculated from their relative distribution between the mitosis observed (phase indices[9]) and within the meristematic cell population (phase numbers[10]).

At 1% concentration, DMSO causes the drop of the mitotic index during the first few h of treatment, eventually reaching a balance after 24 h (Figure 1). No morphological anomaly or atypical mitotic pattern is observed. At 6%, DMSO depresses the mitotic index, which reaches very low levels after 24 h of continuous treatment (Figure 1). The proportion of cells in the various stages of mitosis remains practically unaltered (Figure 2), although the study of the phase numbers shows an effect on the cell cycle (Figure 3). Alongside the depressive effect on mitosis, we observe a number of cytopathological anomalies. Thus, we find patterns similar to those of c-metaphases and multipolar anaphases (Figure 4).

At a concentration of 10%, DMSO affects the mitotic index and eventually causes the death of the cells at about the 6th h (Figure 1), accompanied by a loss of turgescence in the roots and the progressive pulverization of the chromatin. The mitotic patterns previously observed persist, but increased in frequency, together with the appearance of numerous chromatin bridges, and a peculiar contraction of the chromatin during telophase (Figure 4). Finally, at a concentration of 20%, DMSO produces an immediate lethal effect on the roots.

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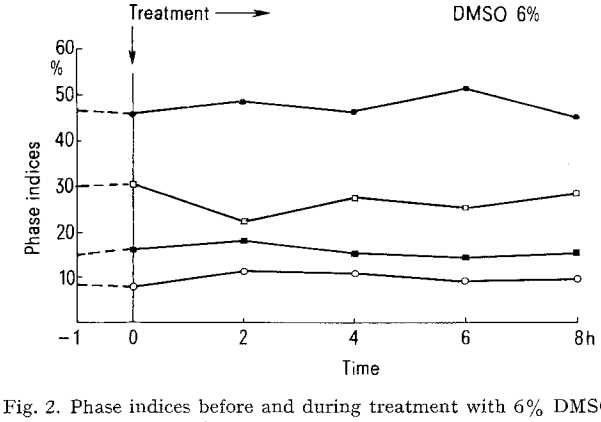

Fig. 2. Phase indices before and during treatment with 6% DMSO. -●- prophase; -□- telophase; -■- metaphase; -○- anaphase.

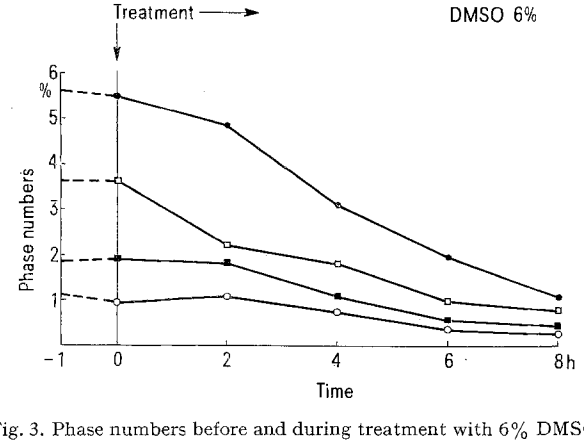

Fig. 3. Phase numbers before and during treatment with 6% DMSO. -●- prophase; -□- telophase; -■- metaphase; -○- anaphase.

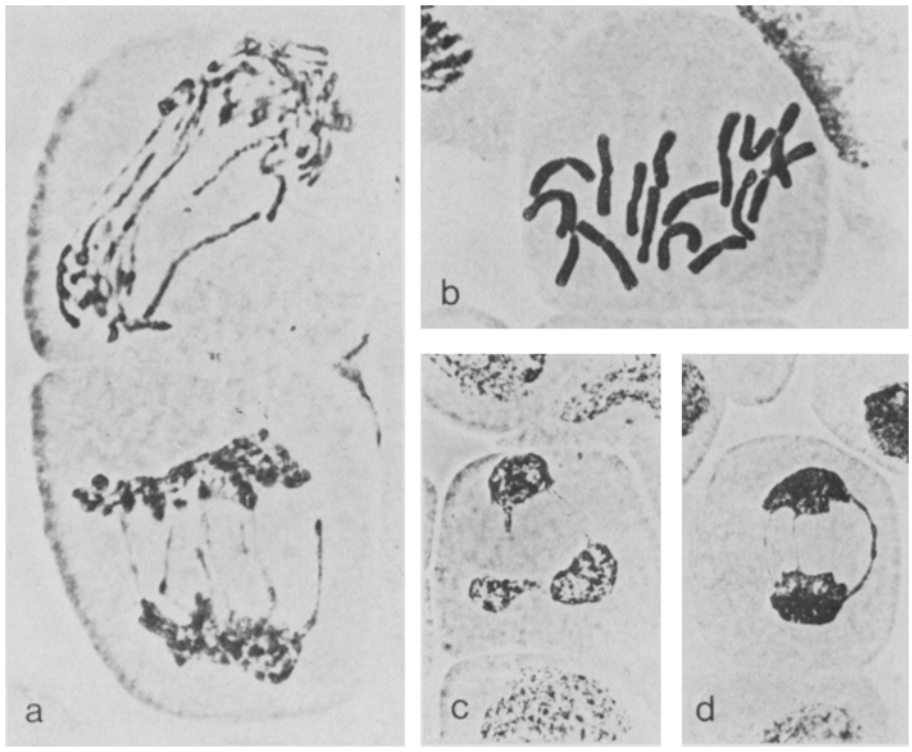

Fig. 4. Root-tip cells during DMSO treatment at 6 and 10% concentrations: a, c, d) cells in anaphase and telophase showing chromatin bridges. b) c-metaphase cell. c) tripolar mitosis.

DMSO is mentioned as being a powerful antimetabolite. Zinc uptake is affected at low concentration[11] but cell metabolism and enzymatic activity is not altered[12,13]. However, although in our study system no morphological changes appear at the concentration of 1%, we observed an effect on mitotic index.

Recent studies suggest that the primary effect of DMSO is to alter the structure and permeability of the cell membrane[14,15] and this would explain its highly lethal effect on the meristematic cells at high concentrations. Most studies dealing with the effects of DMSO at high concentrations conclude by drawing attention to the toxic nature of this compound, which exercises its effect through cell metabolism. Thus, SCHMID[16] showed that, at concentrations above 10%, it stimulated the uptake of zinc, while inhibiting that of Na and Rb.

At a concentration of 10%, DMSO acts upon the metabolism of RNA and proteins, to a large extent inhibiting the uptake and incorporation of ^{14}C-uracil and ^{14}C-methionine in plant tissues[11]. Moreover, at this concentration, the respiration of the cell is inhibited. The specific activity of several enzymes is also blocked at high concentrations[12,13].

The constancy of the phase indices which we have observed, indicates that DMSO does not exert any selective effect on any particular phase of mitosis. The progressively decreased frequency of cells in each phase of mitosis within the meristematic population in a proof that cells are blocked at their entry upon division.

BAJAJ et al.[11] point out that the effect of DMSO is greater, and appears earlier, upon the uptake of O_2 than in the case of proteins and nucleic acids, whence we should conclude that the blocking of metabolism is effected primarily through the respiratory process. On the other hand, LÓPEZ-SÁEZ et al.[17] observed the high degree of dependence of mitotic activity on the oxygen tension of the medium in *Allium* roots. The depressive effect on the mitotic index observed by us might be due to a progressive lack of energy (energy of the phosphate bond, ATP) as a result of a failure in the respiratory mechanism, which would affect both the phenomenon of transcription and protein synthesis.

Bearing in mind the evidence of depolymerizing activity on the part of various c-mitotic agents which have been thoroughly studied[18], the effect observed on the mitotic spindle might be interpreted as due to the linking of DMSO with certain tubular proteins, or to the blockage of some enzymatic system which is necessary for the normal development of the spindle.

And lastly, the frequent anaphase bridges and the condensation of the chromatin would seem to indicate that DMSO acts directly as well as indirectly upon the chromosomal structure.

Resumen. Dimetil sulfóxido, potente antimetabolito, a distintas concentraciones altera significativamente la cinética de poblaciones meristemáticas radicales de *Allium cepa* L., produciendo un cuatro de alteraciones morfológicas y figuras mitóticas atípicas.

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